

AMENDMENTS TO THE CLAIMS

Please amend the claims as follows.

Please cancel claims 17, 18, 77, 78, 85, 97, 112 and 118 to 125, without prejudice or disclaimer.

This listing of claims will replace all prior versions, and listing, of claims in the application:

Claim 1 (previously presented): An isolated or recombinant nucleic acid comprising (a) a sequence having at least 90% sequence identity to SEQ ID NO:26, and encoding a polypeptide having an esterase activity, or, (b) a sequence complementary to (a).

Claim 2 (previously presented): An isolated or recombinant nucleic acid of claim 1, comprising a sequence comprising SEQ ID NO:26 or sequences complementary thereto.

Claim 3 (currently amended): An isolated or recombinant nucleic acid that hybridizes to a nucleic acid comprising (a) a sequence as set forth in having at least 90% sequence identity to SEQ ID NO:26, and encoding a polypeptide having an esterase activity, or (b) sequences complementary to (a), under conditions comprising about 50% formamide at about 37°C to 42°C, 5X SSPE, 0.3% SDS, and 200 ng/ml ~~[[n/ml]]~~ sheared and denatured salmon sperm DNA, and a wash for 30 minutes at room temperature in 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na₂EDTA containing 0.5% SDS.

Claim 4 (currently amended): An isolated or recombinant nucleic acid that hybridizes to a nucleic acid comprising (a) a sequence as set forth in having at least 90% sequence identity to SEQ ID NO:26, and encoding a polypeptide having an esterase activity, or (b) sequences complementary to (a), under hybridization conditions comprising a wash for 30 minutes at room temperature in 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na₂EDTA containing 0.5% SDS, followed by a 30 minute wash in fresh 1X SET at T_m-10°C.

Claim 5 (currently amended): An isolated or recombinant nucleic acid that hybridizes to a nucleic acid comprising (a) a sequence as set forth in ~~having at least 90% sequence identity to~~ SEQ ID NO:26, and encoding a polypeptide having an esterase activity, or (b) sequences complementary to (a), under conditions comprising about 35% formamide at about 35°C to 42°C, 5X SSPE, 0.3% SDS, and 200 ng/ml ~~[[n/ml]]~~ sheared and denatured salmon sperm DNA.

Claims 6 to 15 (canceled)

Claim 16 (currently amended): An isolated or recombinant nucleic acid comprising (a) at least 30 consecutive bases of a sequence as set forth in SEQ ID NO:26 ~~or, at least 30 consecutive bases of a sequence having at least 90% identity to SEQ ID NO:26 and encoding a polypeptide having an esterase activity,~~ or (b) sequences complementary to (a), wherein the nucleic acid can specifically hybridize to an esterase-encoding sequence under hybridization conditions comprising a wash for 30 minutes at room temperature in 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na₂EDTA containing 0.5% SDS, followed by a 30 minute wash in fresh 1X SET at T_m-10°C.

Claims 17 to 19 (canceled)

Claim 20 (currently amended): The isolated or recombinant nucleic acid of claim 1 ~~[[16]]~~, wherein the nucleic acid has a 95% sequence identity to SEQ ID NO:26 ~~is at least about 95%.~~

Claim 21 (previously presented): The isolated or recombinant nucleic acid of claim 20, wherein the sequence identity to SEQ ID NO:26 is at least about 97%.

Claim 22 (previously presented): An isolated or recombinant nucleic acid encoding (a) a polypeptide having an esterase activity and having at least 90% sequence identity to a sequence as set forth in SEQ ID NO:36, or, (b) enzymatically active fragments of (a).

Claim 23 (previously presented): An isolated or recombinant nucleic acid encoding a polypeptide comprising at least 30 consecutive amino acids of a polypeptide having an esterase activity and having at least 90% sequence identity to a sequence as set forth in SEQ ID NO:36.

Claims 24 to 39 (canceled)

Claim 40 (currently amended): A method of producing a polypeptide having an esterase activity comprising introducing a nucleic acid as set forth in claim 1 or claim 3 into a host cell under conditions that allow expression of the nucleic acid to produce a polypeptide.

Claim 41 (currently amended): A method of producing a polypeptide having esterase activity comprising at least 30 amino acids of a sequence as set forth in SEQ ID [[ED]] NO:36 or at least 30 amino acids of a sequence encoded by a nucleic acid as set forth in claim 1, comprising introducing a nucleic acid encoding the polypeptide, operably linked to a promoter, into a host cell under conditions that allow expression of the polypeptide, wherein said polypeptide has esterase activity.

Claim 42 (withdrawn): A method of generating a variant comprising:

obtaining a nucleic acid comprising a sequence as set forth in SEQ ID NO:26, or a sequence as set forth in claim 1, or, sequences complementary thereto, or fragments comprising at least 30 consecutive nucleotides thereof, or fragments comprising at least 30 consecutive nucleotides of the sequences complementary to SEQ ID NO:26; and

modifying one or more nucleotides in said sequence to another nucleotide, deleting one or more nucleotides in said sequence, or adding one or more nucleotides to said sequence.

Claim 43 (currently amended): The method of claim 42, wherein the modifications are introduced by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble

mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturation ~~saturated~~ mutagenesis and any combination thereof.

Claim 44 (withdrawn): The method of claim 42, wherein the modifications are introduced by error-prone PCR.

Claim 45 (withdrawn): The method of claim 42, wherein the modifications are introduced by shuffling.

Claim 46 (withdrawn): The method of claim 42, wherein the modifications are introduced by oligonucleotide-directed mutagenesis.

Claim 47 (withdrawn): The method of claim 42, wherein the modifications are introduced by assembly PCR.

Claim 48 (withdrawn): The method of claim 42, wherein the modifications are introduced by sexual PCR mutagenesis.

Claim 49 (withdrawn): The method of claim 42, wherein the modifications are introduced by *in vivo* mutagenesis.

Claim 50 (withdrawn): The method of claim 42, wherein the modifications are introduced by cassette mutagenesis.

Claim 51 (currently amended): The method of claim 42, wherein the modifications are introduced by recursive ensemble mutagenesis.

Claim 52 (withdrawn): The method of claim 42, wherein the modifications are introduced by exponential ensemble mutagenesis.

Claim 53 (withdrawn): The method of claim 42, wherein the modifications are introduced by site-specific mutagenesis.

Claim 54 (withdrawn): The method of claim 42, wherein the modifications are introduced by gene reassembly.

Claim 55 (currently amended): The method of claim 42, wherein the modifications are introduced by gene site gene site saturation ~~saturated~~ mutagenesis.

Claims 56 to 60 (canceled)

Claim 61 (currently amended): A method for comparing a first sequence to a reference sequence wherein said first sequence comprises ~~[[a)] a nucleic acid sequence as set forth in claim 1 or claim 3 SEQ ID NO:26 or (b) a sequence having at least 70% sequence identity to a nucleic acid sequence as set forth in SEQ ID NO:26, or (c) a sequence complementary to (a) or (b), or, (d) a polypeptide sequence as set forth in SEQ ID NO:36 or (e) a sequence having at least 70% sequence identity to a nucleic acid sequence as set forth in SEQ ID NO:26,~~ the method comprising the following steps:

reading the first sequence and the reference sequence through use of a computer program which compares sequences; and

determining differences between the first sequence and the reference sequence with the computer program.

Claim 62 (withdrawn): The method of claim 61, wherein determining differences between the first sequence and the reference sequence comprises identifying polymorphisms.

Claim 63 (currently amended): A method for identifying a feature in ~~[[a)] a sequence as set forth in claim 1 or claim 3 SEQ ID NO:26 or, (b) sequences having at least 70% sequence identity to a nucleic acid sequence as set forth in SEQ ID NO:26, or (c) a sequence complementary to (a) or (b), or, (d) a polypeptide sequence as set forth in SEQ ID NO:36 or (e) having at least 70%~~

~~sequence identity to a nucleic acid sequence as set forth in SEQ ID NO:26~~, the method comprising the following steps:

reading the sequence through the use of a computer program which identifies features in sequences; and
identifying features in the sequences with the computer program.

Claim 64 (canceled)

Claim 65 (withdrawn): A method of catalyzing the hydrolysis of an ester comprising contacting a sample containing an esterase with a polypeptide encoded by a sequence as set forth in claim 1 under conditions which facilitate the hydrolysis of the ester.

Claim 66 (canceled)

Claim 67 (currently amended): A nucleic acid probe for isolation or identification of esterase genes comprising an oligonucleotide at least 30 nucleotides in length and having an area of at least 30 contiguous nucleotides of ~~(a) a sequence having at least 90% sequence identity to a~~ nucleic acid as set forth in SEQ ID NO:26 or (b) a sequence complementary to (a), wherein the nucleic acid probe can specifically hybridize to an esterase-encoding sequence under hybridization conditions comprising a wash for 30 minutes at room temperature in 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na₂EDTA containing 0.5% SDS, followed by a 30 minute wash in fresh 1X SET at T_m-10°C.

Claim 68 (previously presented): The probe of claim 67, wherein the oligonucleotide comprises DNA or RNA.

Claims 69 to 79 (canceled)

Claim 80 (original): The probe of claim 67, wherein the probe further comprises a detectable isotopic label.

Claim 81 (original): The probe of claim 67, wherein the probe further comprises a detectable non-isotopic label selected from the group consisting of a fluorescent molecule, a chemiluminescent molecule, an enzyme, a cofactor, an enzyme substrate, and a hapten.

Claim 82 (currently amended): A nucleic acid probe for isolation or identification of esterase genes comprising an oligonucleotide at least about 20 nucleotides in length and having an area of at least 20 contiguous nucleotides of a sequence ~~(a) having at least 90% sequence identity to a nucleic acid~~ as set forth in SEQ ID NO:26, or, (b) its complementary sequence.

Claims 83 to 87 (canceled)

Claim 88 (currently amended): A method for modifying small molecules, comprising mixing a polypeptide encoded by a polynucleotide of claim 1 or claim 3 ~~enzymatically active fragments~~ thereof with a small molecule to produce a modified small molecule.

Claim 89 (withdrawn): The method of claim 88 wherein a library of modified small molecules is tested to determine if a modified small molecule is present within the library which exhibits a desired activity.

Claim 90 (withdrawn): The method of claim 89 wherein a specific biocatalytic reaction which produces the modified small molecule of desired activity is identified by systematically eliminating each of the biocatalytic reactions used to produce a portion of the library, and then testing the small molecules produced in the portion of the library for the presence or absence of the modified small molecule with the desired activity.

Claim 91 (withdrawn): The method of claim 90 wherein the specific biocatalytic reactions which produce the modified small molecule of desired activity is optionally repeated.

Claim 92 (withdrawn): The method of claim 90 or 91 wherein (a) the biocatalytic reactions are conducted with a group of biocatalysts that react with distinct structural moieties found within

the structure of a small molecule, (b) each biocatalyst is specific for one structural moiety or a group of related structural moieties; and (c) each biocatalyst reacts with many different small molecules which contain the distinct structural moiety.

Claims 93 to 97 (canceled)

Claim 98 (currently amended): A vector comprising a nucleic acid as set forth in claim 1 or claim 3 [[23]].

Claim 99 (previously presented): The vector of claim 98, wherein the vector comprises a viral particle, a baculovirus, a phage, a plasmid, a cosmid, a fosmid, a bacterial artificial chromosome, a viral DNA or a P1-based artificial chromosome.

Claim 100 (currently amended): A host cell comprising a nucleic acid as set forth in claim 1 or claim 3 [[23]].

Claim 101 (previously presented): The host cell of claim 100 comprising a eukaryotic cell or a prokaryotic cell.

Claim 102 (previously presented): The host cell of claim 101 comprising a plant cell, a mammalian cell, a fungal cell, a bacterial cell, a yeast cell or an insect cell.

Claims 103 to 106 (canceled)

Claim 107 (currently amended): The isolated or recombinant nucleic acid of claim 1 or claim 3, wherein the esterase activity comprises catalysis of ~~a transesterification reaction~~ hydrolyzing ester groups to organic acids and alcohols.

Claim 108 (currently amended): The isolated or recombinant nucleic acid of claim 1 or claim 3, wherein the esterase activity comprises hydrolase activity ~~catalysis of an acidolysis reaction~~.

Claim 109 (currently amended): The isolated or recombinant nucleic acid of claim 1 or claim 3, wherein the esterase activity functions at ~~extreme~~ temperatures above 100°C, or, below 0°C.

Claim 110 (previously presented): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 35 nucleotides in length.

Claim 111 (previously presented): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 40 nucleotides in length.

Claim 112 (canceled)

Claim 113 (previously presented): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 50 nucleotides in length.

Claim 114 (previously presented): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 75 nucleotides in length.

Claim 115 (previously presented): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 100 nucleotides in length.

Claim 116 (previously presented): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 150 nucleotides in length.

Claim 117 (previously presented): The nucleic acid probe of claim 67, wherein the oligonucleotide is at least 200 nucleotides in length.

Claims 118 to 125 (canceled)

Claim 126 (new): The isolated or recombinant nucleic acid of claim 1 or claim 3, wherein the polypeptide retains esterase activity in an environment comprising a pH of greater than pH 11.